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EXPERIMENTAL GAS GANGRENE

THE PROTECTION BY ANTISERUM AND ANTISERUM MIXTURES

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As Bull's antitoxin against B. welchii[1] was being tried out as a prophylactic and therapeutic agent in certain sectors of the British Army during the spring and summer of 1918, it seemed of interest to verify and if possible extend the experimental basis for serum treatment in gas gangrene. The claims for the potency of the antitoxin rested on experiments carried out with pure strains of B. welchii, and no attempt had been made to reproduce more accurately the complex bacteriologic factors found in practically all war wounds.

It is now generally known, at least in war conditions, that the clinical picture called gas gangrene, is almost never due to a pure B. welchii infection, but to a combined action of a variety of anaerobic organisms. It is further held by some authors that although B. welchii is almost universally present in the lesions of gas gangrene, it is not the most important factor in the etiology of the disease,[2] and that the presence of two other anaerobic bacilli, B. edematiens and "vibrion septique," constitute a much more serious bacteriologic invasion.

The following series of experiments were planned with the idea of simulating as nearly as possible the bacteriologic conditions characteristic of war wounds, and to determine the efficacy of the different serums in combatting a mixed anaerobic infection in which the anaerobes were present in different combinations. The experiments were planned to show:

1. The relative value of B. welchii antitoxin and perfringens antimicrobial serum.*

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* B. perfringens and B. welchii are two names for the same organism, the former being current among the French investigators, the latter among the English and American. The B. perfringens antimicrobial serum is produced by the inoculation of dead and living cultures of the organism, whereas the B. welchii antitoxin is produced by the inoculation of sterile filtrates of the cultures.

[1] Jour. Exp. Med., 1917, 26, p. 603.

[2] Sacquepée, Presse méd., 1918, 26, p. 197. Weinberg et Seguin, Compt. rend. de la Soc. biol., 1915, 727.

2. The value of various antiserums both singly and combined against edematous exudate produced by the inoculation of several anaerobic organisms.

3. The protective value of various antiserums both singly and combined against mixed anaerobic infections.

TABLE 1

EXPERIMENT 1, INOCULATION WITH 2 M. L. D. OF B. WELCHII

Strains of Bacilli	Antiserum in C C	Results
B. perfringens	Antiperfringens serum 4	Died within 24 hours
	Antiperfringens serum 4	Developed necrotic area in 5 days
	Antiperfringens serum 4	Animal lived, no local reaction
Lister B	Antiperfringens serum 4	Died within 48 hours
	Antiperfringens serum 4	Animal lived, no local reaction
	Antiperfringens serum 4	Animal lived, no local reaction
1877	Antiperfringens serum 4	Died within 24 hours
	Antiperfringens serum 4	Animal lived, no local reaction
	Antiperfringens serum 4	Animal lived, no local reaction
779	Antiperfringens serum 4	All three died within 24 hours
	Antiperfringens serum 4	
	Antiperfringens serum 4	
B. perfringens	B. welchii antitoxin 2	Developed necrotic area in 48 hours
	B. welchii antitoxin 1.3	Developed necrotic area in 48 hours
	B. welchii antitoxin 1	Died in 24 hours
Lister B	B. welchii antitoxin 1.8	Died in 24 hours
	B. welchii antitoxin 1.3	Developed necrotic area in 6 days
	B. welchii antitoxin 1	Died within 48 hours
1877	B. welchii antitoxin 2	Died within 48 hours
	B. welchii antitoxin 1	Animal lived, no local reaction
	B. welchii antitoxin 1	Animal lived, no local reaction
799	B. welchii antitoxin 1.5	Died within 5 days
	B. welchii antitoxin 1	Died within 24 hours
	B. welchii antitoxin 1	Developed necrotic area in 48 hours
B. perfringens	Normal horse serum 1.7	All died within 24 hours
	Normal horse serum 1.2	
	Normal horse serum 1	
Lister B	Normal horse serum 2	
	Normal horse serum 1.1	
	Normal horse serum 1	
1877	Normal horse serum 1.7	
	Normal horse serum 1	
	Normal horse serum 1	
779	Normal horse serum 1.9	
	Normal horse serum 1.3	
	Normal horse serum 1	
B. perfringens	Culture controls	All died within 24 hours
Lister B		
1877		
779		

EXPER. 1

Twelve guinea-pigs were inoculated subcutaneously with B. welchii antitoxin, 0.5 c c per 100 gm. body weight of animal.

The same number of animals were inoculated subcutaneously with 4 c c of antiperfringens serum, irrespective of body weight.

Four days later the animals were inoculated intramuscularly with two minimum lethal doses (M. L. D.) of living, virulent cultures of B. welchii, a minimum lethal dose being the smallest amount of an eighteen hour culture killing a 300 gm. guinea-pig in 48 hours.

As seen from table 1, the animals were inoculated in groups of three with each of four cultures, each animal receiving the same dosage according to weight. On account of the shortage of animals only two guinea-pigs were used for each culture control, as this was considered and proved to be sufficient.

The culture control animals, and those receiving cultures and normal horse serum, died in less than 24 hours.

Of the animals receiving cultures and antiperfringens serum, five died in less than 24 hours, and one in less than 48 hours. Of the six that lived, only one gave any reaction to the culture. This animal developed an extensive necrosis of the abdominal surface.

Of the animals receiving cultures and B. welchii antitoxin, three died within 24 hours, two within 48 hours, and one within 5 days. Of the six remaining animals, three developed within 48 hours and one within 6 days, a liquefactive necrosis over the abdominal surface, with marked swelling of the inoculated leg. Only two of the animals failed to develop any evidence of the inoculation.

DISCUSSION

From these experiments it would seem that some protection is afforded both by B. welchii antitoxin and perfringens antimicrobial serum against the inoculation of pure cultures of B. welchii.

The fact that necrotic areas occurred at the site of inoculation in a much smaller percentage of the animals inoculated with antiperfringens serum than in those inoculated with B. welchii antitoxin, seems to indicate that the antibodies contained in the antimicrobial serum are more effective than the antitoxic principle contained in the antitoxin in preventing circumscribed proliferation of the organism, thus eliminating even local necrotic areas.

The protection established by antiperfringens serum, therefore, seems more clean-cut in its results than the protection by B. welchii antitoxin, but this apparent difference may be due to the low antitoxic content (700 units per c c) of the lot of antitoxin used.

At a later date, Major Bull personally conducted a series of experiments in this laboratory with a fresh lot of antitoxin brought over by him from America. In these experiments in contrast to the writer's results, 100% protection was afforded instead of only 50%. The following conditions differed in the two experiments: the antitoxin used by Major Bull was dated Dec. 12, 1917, with 1,900 units per c c, the lot used by the writer Nov. 28, 1917, 700 units per c c. The difference in the dates of the two lots amounts to less than a month, but the later antitoxin contained 2.71 times as many antitoxic units per c c as the earlier lot. Notwithstanding the higher potency of the antitoxin used by Major Bull, the same number of M. L. D. (2) was given in both experiments. Furthermore, in Major Bull's experiment, although the lot of antitoxin was of higher potency than the lot used by the writer, the antitoxin was injected 48 hours before the inoculation of the cultures, instead of allowing four days to elapse as in the writer's experiment. It is impossible to know to which of these three factors: the difference in the age of the two lots, the difference in the potency of the two lots, the difference in the time between the inoculation of the antitoxins and the injection of the cultures, to ascribe the greater efficacy of the second lot of antitoxin, without more detailed work, but it would seem probable that the higher antitoxic content of the second lot might prove to be the most important factor.

From the results of Major Bull's experiment it seems apparent that an antitoxin of such potency can be produced as to give complete protection in the case of a pure B. welchii infection. The careful bacteriologic examination of war wounds, however, has shown that B. welchii almost always occurs in association with other anaerobic bacilli, and the next experiment was undertaken to determine the efficacy of the B. welchii antitoxin and the antiperfringens serum in the presence of combinations of anaerobic bacilli characteristic of war gas gangrene.

EXPER. 2

The production of edema in the development of gas gangrene is a very frequent occurrence. McNee and Dunn[3] were the first investigators to suggest by a series of pathologic sections at various levels of an infected muscle, that the spread of the invading organisms is not preceded, as was commonly supposed, by the penetration of gas and the exertion of pressure on fresh tissues, but that the anaerobic bacteria followed in the path of a fluid or edema which was forced along the muscle fibers in advance of the infection.

This edema seemed to contain toxic principles which the body fluids were, in the majority of cases, powerless to neutralize, and which affected the fresh muscle tissue in such a way that the anaerobic bacteria, associated with gas gangrene could multiply freely in it.

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[3] Br. Med. Jour., 1917, 1, p. 727.

From a prophylactic and particularly from a therapeutic point of view, it was essential that a really effective serum should be able to counteract the toxic action of the edema which always prepared the new "terrain," and without which the anaerobes could not make their way in fresh tissue.

TABLE 2

Exper. 2, Inoculation with 2 M. L. D. of Fluid from Guinea-Pigs Injected with Cultures of B. Welchii, Vibrion Septique, and B. Sporogenes

Antiserums	Antiserum in C C	Results
Antiperfringens serum	2.4	Died within 48 hours
	2	
	1.7	
B. welchii antitoxin	2.4	Died within 48 hours
	1.7	
	1.7	
Antivibrion septique	2.4	Animal lived, no local reaction
	2	Died within 3 days
	1.8	Animal lived, no local reaction
Antiperfringens serum and antivibrion septique, equal parts	2.5	Animal lived, no local reaction
	1.7	Animal lived, no local reaction
	1.7	Died in 55 hours
B. welchii antitoxin and antivibrion septique, equal parts	2.4	Animal lived, no local reaction
	2	Animal lived, no local reaction
	1.8	Died within 24 hours
Normal horse serum	2.4	Died within 24 hours
	2	
	1.6	
Control animals	...	Died within 24 hours
	...	
	...	

The next series of experiments were, therefore, carried out with edematous fluid collected from animals inoculated with different combinations of anaerobes in the following way:

Virulent 18-hour meat cultures of the following organisms: B. welchii, vibrion septique, and B. sporogenes, were mixed in equal quantities, and guinea-pigs inoculated intramuscularly with 5 c c of the mixture. On the death of the animals the edematous fluid was collected and pooled and the M. L. D. established. The edematous fluid was kept on ice, and no appreciable change in the virulence of the fluid was observed during the course of the experiment. The examination of the edematous fluid showed the presence of the three originally inoculated organisms.

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* As there still exists some confusion concerning the culture known as vibrion septique, it may be well to add that the culture used throughout these experiments was the non-proteolytic, highly pathogenic organism corresponding to the classification of the British Medical Research Committee.

Guinea-pigs ranging in weight from 300-400 gm. were inoculated subcutaneously as follows: 0.5 c c per 100 gm. weight.

Three with B. welchii antitoxin.
Three with antiperfringens serum.
Three with antivibrion septique serum.
Three with equal parts B. welchii antitoxin and antivibrion septique serum.
Three with equal parts antiperfringens serum and antivibrion septique serum.
Three with normal horse serum.

Four days later the animals were all inoculated into the thigh muscle with two M. L. D. of edema fluid. Three control guinea-pigs were also inoculated with the fluid alone.

The control animals and those receiving normal horse serum died within 24 hours.

The animals receiving B. welchii antitoxin and antiperfringens serum died within 24 and 48 hours.

Of the animals receiving B. welchii antitoxin and antivibrion septique serum mixture, one died within 24 hours. Of those receiving the antiperfringens serum and the antivibrion septique serum mixture, one died in 55 hours, while those animals receiving antivibrion septique serum alone, one died in 3 days (table 2).

In the same manner, animals were inoculated with B. welchii antitoxin, with antiperfringens serum. Four days later, the same animals were injected with two M. L. D. of edema produced by a mixture of virulent strains of B. welchii, B. edematiens and B. histolyticus; three control animals were also inoculated at this time.

All the animals died within 48 hours (table 3). Necropsies as in the preceding experiment were made on a large proportion of the animals and in each case all the organisms present in the originally inoculated edematous fluid were isolated.

TABLE 3

EXPER. 2, INOCULATION OF 2 M. L. D. OF FLUID FROM GUINEA-PIGS INJECTED WITH B. WELCHII, B. EDEMATIENS, B. HISTOLYTICUS

Antiserums	Antiserum in C C	Results
Antiperfringens serum	2.3 2 1.9	All died within 48 hours
B. welchii antitoxin	2.3 1.8 1.6	
3 control animals	...	

DISCUSSION

The experiments in tables 2 and 3 show no evidence that any protection was afforded by either antiperfringens serum or Bull's antitoxin against a mixed anaerobic infection in which B. welchii was associated in each case with two other anaerobes occurring commonly in war wounds.

Neither B. sporogenes nor B. histolyticus in pure culture are able to kill unless given in very large doses, whereas the pathogenic powers of both B. edematiens and vibrion septique are well known.

The death of the animals (table 2) can probably be ascribed to the presence of vibrion septique, and (in table 3) to B. edematiens. This point is brought out in table 2 by the fact that antivibrion septique serum alone, in spite of the presence of B. welchii in the combination of the infecting organisms, gives protection in 62.3%. The potency of the vibrion septique serum is further shown by the fact that the animals which were given equal mixtures of vibrion septique serum and antiperfringens serum, and vibrion septique and Bull's antitoxin — thus receiving only half of the prescribed protective dose — were also protected in 62.3% of the cases.

Unfortunately, at the time this experiment was done, no B. edematiens serums was available, so that the power of B. edematiens serum alone to protect against a mixed anaerobic infection containing B. welchii as well as B. edematiens was not tested at this point.

It seems apparent that neither antiperfringens serum nor Bull's antitoxin afford any protection when other pathogenic anaerobic organisms incident to war wounds are present together with B. welchii. These two serums must, therefore, be considered of little if any practical value in the treatment of wounds, since B. welchii, in almost every instance, occurs in association with other anaerobes.

On the other hand, vibrion septique serum shows distinct evidence that it is applicable in a practical way to war wounds, because it is able to combat alone a mixed infection in which B. welchii and other less virulent anaerobes are present as well as vibrion septique.

EXPER. 3

Tweny-four-hour cultures of the following organisms were mixed in equal parts and the 48-hour M. L. D. of the mixtures established.

Mixture 1	Mixture 2	Mixture 3
B. welchii	B. welchii	B. welchii
Vibrion septique	B. edematiens	B. bellonensis
B. sporogenes	B. sporogenes	B. sporogenes

It will be noticed that B. welchii and B. sporogenes are present in each of the three mixtures. This was thought advisable as these two organisms are present in a large percentage of the wounds and the desire was to make the experiment as comparable to the condition of the wound when it reaches the evacuation hospital as was possible.

Three guinea-pigs were each inoculated subcutaneously with the following antiserum, and antiserum mixtures, 0.5 c c per 100 gm. body weight.

TABLE 4

EXPER. 3, INOCULATION OF 2 M. L. D. OF CULTURE MIXTURES

Antiserums	Antiserums in C C	Culture Mixtures	Results
B welchii antitoxin..........	3.5 3 3	1	All found dead within 48 hours
Antiperfringens serum........	2 2 2	1	All found dead within 24 or 48 hours
Antivibrion septique serum...	2 2 2	1	Animals lived, no local reaction
B. welchii antitoxin and vibrion septique serum	3.3 2.9 2.8	1	Animals lived, no local reaction
Antiperfringens and antivibrion septique serum	3.3 2.8 2.7	1	Animals lived, no local reaction
B. welchii antitoxin..........	3.2 3 3	2	All found dead within 48 hours
Antiperfringens serum........	3.3 2.8 2.8	2	All found dead within 48 hours
Antiedematous serum	3.3 2.9 2.8	2	Animals lived, no local reaction
B. welchii antitoxin and antiedematiens serum	3.3 3 3	2	Animals lived, no local reaction
Antiperfringens and antiedematiens serum	3.3 2.6 2.5	2	Animals lived, no local reaction
B. welchii antitoxin..........	3.2 2.7 2.7	3	All found dead within 48 hours
Antiperfringens serum........	3.3 2.8 2.7	3	All found dead within 48 hours
Antibellonensis serum........	3.3 2.7 2.7	3	Animals lived, no local reaction
B. welchii antitoxin and antibellonensis serum	3.1 2.9 2.8	3	Animals lived, no local reaction
Antiperfringens serum and antibellonensis serum	3.1 2.9 2.8	3	Animals lived, no local reaction
3 control animals............	...	3	Found dead within 24 hours

The animals subsequently receiving mixture 1 were inoculated as follows:
- Three with B. welchii antitoxin.
- Three with antiperfringens serum.
- Three with anti-vibrion serum.
- Three with B. welchii antitoxin and antivibrion septique serum, equal parts.
- Three with antiperfringens serum and antivibrion septique serum, equal parts.

The animals subsequently receiving mixture 2 were inoculated as follows:
- Three with B. welchii antitoxin.
- Three with antiperfringens serum.
- Three with antiedematiens serum.
- Three with B. welchii antitoxin and antiedematiens serum, equal parts.
- Three with antiperfringens serum and antiedematiens serum, equal parts.

The animals subsequently receiving mixture 3 were inoculated as follows:
- Three with B. welchii antitoxin.
- Three with antiperfringens serum.
- Three with antibellonensis serum.
- Three with B. welchii antitoxin and antibellonensis serum, equal parts.
- Three with antiperfringens serum and antibellonensis serum, equal parts.

Four days later, the animals were injected deep into the thigh muscles with 2 M. L. D. of the various culture mixtures. Three control animals received intramuscularly inoculations of 2 M. L. D. of cultures 1, 2 and 3, respectively (table 4). On account of the shortage of guinea-pigs, and previous experiments having shown that no protection was afforded by normal horse serum, it was not deemed necessary to make experiments with it.

DISCUSSION

In no instance was any appreciable protection established by either B. welchii antitoxin or the antiperfringens serum.

In all instances when antivibrion septique, anti-edematiens, or antibellonensis serums were used, either alone or mixed in equal amounts with B. welchii antitoxin or antiperfringenes serum, 100% protection was afforded in the combination containing the specific organism.

As in Exper. 2, the effectiveness is again demonstrated of antivibrion septique serum alone to combat a mixed infection containing B. welchii as well as vibrion septique, in this instance by the inoculation of equal amounts of broth cultures, instead of the mixed edema. In this case also the potency of vibrion septique serum when diluted with equal quantities of either Bull's antitoxin or antiperfringens serum, is brought out.

In the same way, anti-edematiens and antibellonensis serums are effective against combinations containing, respectively, as the two most pathogenic factors, B. edematiens and B. welchii, and B. bellonensis and B. welchii. These two latter serums are also potent when diluted with equal quantities of Bull's antitoxin or antiperfringens serum.

No evidence can be deduced from these experiments to show that either antiperfringens serum or Bull's antitoxin should be used singly for prophylactic purposes in the treatment of war wounds. These serums would seem of value only in a bacteriologic condition (namely a *pure* infection of B. welchii), which occurs so rarely as to be negligible.

The evidence, on the other hand, in favor of using antivibrion septique, anti-edematiens, antibellonensis serums in a practical way is striking. All three of these serums are able to protect against a mixed infection containing B. welchii plus the specific organism.

B. edematiens and B. bellonensis are considered to be the same organism by the majority of writers, so that one serum could be prepared for these two organisms. Vibrion septique differs sharply from B. edematiens or B. bellonensis, and a separate serum would have to be made.

Whether the addition of either antiperfringens or Bull's antitoxin to vibrion septique and B. edematiens (B. bellonensis) serums, is desirable in treating a mixed infection, or whether these two latter serums would be just as effective alone, is still to be determined. Perhaps some light can be brought on this question from the clinical data, as well as from animal experimentation.

Since it often requires a long and difficult bacteriologic technic to identify vibrion septique and B. edematiens (B. bellonensis) it would seem advantageous to prepare a mixed serum from these two organisms and give it without waiting for the laboratory report.

The careful work of a few writers has shown that either vibrion septique, B. edematiens (B. bellonensis) are present, although frequently overgrown, in practically every case of gas gangrene. It would seem, therefore, in preparing serums to combat these two most pathogenic anaerobic bacilli, the danger from the other anaerobic bacteria becomes a minor question.

Undoubtedly, the significance of B. welchii in the lesions of gas gangrene, insofar as it had been observed in civilian practice, has been exaggerated, and it was not until the importance of vibrion septique and B. edematiens (B. bellonensis) was recognized in the conditions of actual warfare that a sound basis for the serum treatment of gas gangrene was established.

CONCLUSIONS

When vibrion septique and B. edematiens (B. bellonensis) are present in mixed infections the prophylactic use of the specific serums even when diluted by another serum, is effective.

Neither the B. welchii antitoxin nor the B. welchii (perfringens) antimicrobial serum are of any practical value in the prophylaxis of gas gangrene caused by a mixed infection due to several anaerobic bacilli, such as is commonly found in war wounds today. No accurate investigations of the amount of antitoxin contained in the antiperfringens (B. welchii) serum were made, and it is therefore impossible to say whether the protection afforded in pure B. welchii infections by the antimicrobial serum was due to the antitoxic principle contained in it, or to other antibodies present, or to a combination of the two. Further studies will be necessary to determine whether a serum of the highest potency can be produced by the inoculation of a sterile filtrate, producing a strict antitoxin, or by the inoculation of whole cultures, producing an antimicrobial serum.

NOTE.—The culture known as Lister B was one sent by Muriel Robertson and was identical with the one used by Major Bull in his experiments, at the Lister Institute, with the B. welchii antitoxin from the Rockefeller Institute. The culture known as B. perfringens was sent by M. Weinberg of the Pasteur Institute in Paris. This culture is the "souche toxique" used by M. Weinberg in the production of his antiperfringens serum. The culture known as 1877 was isolated by the writer in this hospital from a deep buttock wound. The culture known as 779 was a stock laboratory culture source unknown. The culture of vibrion septique came from M. Veillon, the culture of B. bellonensis from M. Sacquepee, the cultures of B. edematiens and B. histolyticus from M. Weinberg, all of the Pasteur Institute in Paris. The B. welchii antitoxin was obtained from the Rockefeller Institute. All the remaining serums were obtained from the Pasteur Institute.